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Prevalence of Enteric Viruses in Human Immunodeficiency Virus Seropositive Patients in Venezuela

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The prevalence of enteric viruses associated with gastroenteritis was determined in 125 stool samples from patients infected with the human immunodeficiency virus (HIV), with or without diarrhea. Diagnostic assays included enzyme immunoassays for the identification of rotavirus, adenovirus, and Norwalk virus; polyacrylamide gel electrophoresis for atypical rotaviruses and picobirnaviruses and polymerase chain reaction for astrovirus. Enteric viruses were detected in 6.4% (8 of 125) of the stools collected: five (4.0%) samples positive for adenoviruses, and three (2.3%) samples positive for picobirnaviruses were detected. No rotavirus, astrovirus, or Norwalk virus were observed. Only one of the viruses identified (adenovirus) was found in a sample from a patient with diarrhea. Viruses were detected in 10% of the patients with AIDS, 14% of the symptomatic patients, and none of the asymptomatic persons. These results do not support a major role for enteric viruses in the diarrhea suffered by HIV-infected patients. ***J. Med. Virol. 55:288–292, 1998.***

KEY WORDS: diarrhea; human stool; AIDS; viral pathogens; cryptosporidia

INTRODUCTION

Acute and chronic diarrheas are common complications in human immunodeficiency virus-infected patients: nearly 60% of patients with AIDS in industrialized countries and up to 90% in developing countries suffer from diarrheal disease [Smith, 1992; Mayer and Wanke, 1994]. Epidemiological studies have identified enteric protozoa, atypical mycobacteria, and cytomegalovirus as important etiological agents associated with these diarrheal illness [Sharpstone and Gazzard, 1996]. In addition, HIV itself is also believed to cause intestinal symptoms [Nelson et al., 1988].

The role of pathogenic enteric viruses associated with gastroenteritis [Shaw, 1993; Burns and Greenberg, 1994; Bass, 1996] in causing diarrheal disease in patients infected with HIV is not clear. While some studies detected enteric viruses more frequently in fecal specimens from patients with diarrhea than from those without diarrhea [Cunningham et al., 1988; Albrecht et al., 1993; Grohmann et al., 1993; Schmidt et al., 1996], other studies have failed to show this association [Laughon et al., 1988; Kaljot et al., 1989; Jannof et al., 1991; Thea et al., 1993; Durepaire et al., 1995; Khoo et al., 1995]. Moreover, while studies conducted in the United States have rarely detected rotaviruses [Laughon et al., 1988; Kaljot et al., 1989; Grohmann et al., 1993], these agents were commonly found elsewhere [Cunningham et al., 1988; Albrecht et al., 1993; Thea et al., 1993], suggesting the existence of geographic variation in the viruses infecting these patients [Mayer and Wanke, 1994; Wanke, 1994].

The epidemiological and overall role of enteric viruses in the pathogenesis of AIDS-related diarrhea needs to be more firmly established [Mayer and Wanke, 1994]. Studies in developing countries are particularly lacking. In the present work, we studied 125 stool samples from HIV-infected patients with noncommercially available assays to determine the prevalence of common and novel enteric viruses, and their relationship to diarrhea and to the stage of HIV infection.

PATIENTS AND METHODS

Study Population

Stool samples from 125 HIV seropositive patients (one stool sample per patient) were collected between March 1996 and March 1997 in five hospital centers from Caracas, Venezuela.

To examine the association between viral agents and

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diarrhea, we compared the rates of viral detection in specimens obtained from patients who had diarrhea (case patients, n = 41) with those in specimens obtained from patients who were without diarrhea for at least three months previous to sample collection (controls, n = 84). Diarrhea was defined as the occurrence of three or more bowel movements within a 24-hour period, with decrease in stool consistency (watery or liquid stools). Samples from patients with diarrhea were collected within 72 hours after the onset of symptoms. Case and control patients were matched by sex and age. The mean age of the case patients was 38.9 (range: 20–61 years); that for the control group was 35.2 (range: 19–62). The male-to-female ratio was 13:1 for both groups. Virus detection rates were compared with clinical and CD4+ T-cell categories (1993 classification system, Centers for Disease Control, Atlanta, GA) in 96 (77%) of the 125 for whom this information was available. Among these were 39 AIDS patients, 28 symptomatic patients, and 29 asymptomatic individuals. Fifty six (58%) of the 96 patients had CD4+ T-cell counts of <200/μl.

Fecal Specimens

Samples were stored at −20°C until use. Fecal suspensions, 10% in TNC (10-mM Tris, pH 7.4, 87-mM NaCl, 10-mM $CaCl_2$) buffer, were clarified by low speed centrifugation.

Aliquots of the supernatant were directly tested for group A rotavirus, adenovirus hexon group antigen, and Norwalk virus by sandwich enzyme immunoassays (EIA). Group A rotavirus EIA was performed according to Liprandi et al. [1986]. The adenovirus EIA was performed using a polyclonal, goat antiadenovirus serum as a capture and guinea pig antiadenovirus serum as the detection system. Adenovirus EIA reagents were kindly provided by Dr. Jose Paulo Leite (Instituto Oswaldo Cruz, Rio de Janeiro, Brasil). Positive results for adenovirus were confirmed by a commercial latex agglutination test (Diarlex, Orion Diagnostica, Espoo, Finland) and negative staining electron microscopy. Norwalk virus EIA was performed as described previously [Graham et al., 1994] using reagents kindly provided by Dr. Mary K. Estes (Baylor College of Medicine, Houston, TX).

Approximately 0.5-ml aliquots of the supernatant were extracted for nucleic acids according to the method of Boom et al. [1990]. Extracted nucleic acids were tested for all serotypes of human astrovirus by reverse transcription-polymerase chain reaction (RT-PCR) according to the method of Jonassen et al. [1995]. Tissue-culture-grown astrovirus, serotype 1, was tested in parallel as positive control. Extracted nucleic acids were also tested by PAGE [Laemmli, 1970] for atypical rotavirus and picobirnavirus (PBV). To confirm the results for PBV, new clarified fecal suspensions from the specimens were concentrated by ultracentrifugation at 110,000 xg for two hours, extracted for nucleic acids, digested with DNase I, and analyzed by PAGE.

A subset of 59 of the stool samples collected, 31 from patients with diarrhea and 28 from patients without diarrhea, were examined for *Cryptosporidium spp.* using modified acid-fast Ziehl-Neelsen stain (Merck, Darmstadt, Germany). Oocysts from selected samples were purified by ether–water concentration according to Bukhari and Smith [1995]. The pellet containing the oocysts was resuspended in 1 ml of distilled water and processed for PAGE analysis after nucleic acid extraction.

Statistical Analysis

The rate of virus detection in the specimens from patients in different CD4+ T-cell categories, as well as from patients in clinical asymptomatic categories (A1, A2, A3) and patients in symptomatic and AIDS categories (B1–B3 and C1–C3), were compared for significance ($P \leq 0.05$) with Fisher's exact test (one tailed).

RESULTS

Enteric viruses were detected in 8 (6.4%) of the 125 stool samples collected. Two different viruses were identified, five (4.0%) samples were positive for adenovirus and three (2.4%) were positive for PBV. Three of the five samples positive for adenovirus by ELISA were confirmed by at least one other method; nonetheless, the other two samples were consistently positive by ELISA. The electrophoretic profiles of the three PBV isolated are shown in Figure 1. The genomic segments showed some variation in their electrophoretic mobility, but within a narrow range. All of the viruses detected, except one adenovirus, were found in samples from patients without diarrhea ($P > 0.05$). No rotavirus, Norwalk virus, or astrovirus were detected.

Clinical and CD4+ T-cell categories were associated with virus excretion rates for 77% of the patients. This information was available for all the patients excreting virus. A trend to higher virus detection rates in patients with lower CD4+ T-cells count was observed (Table I); seven out of eight patients excreting enteric viruses showed CD4+ T-cell count of <200/μl. This trend was not significant though ($P = 0.0806$). The trend was stronger when the clinical categories of the patients were considered. Virus was detected in 10% (4 of 39) of the patients with AIDS, 14% (4 of 28) of the symptomatic patients, and none (0 of 29) of the asymptomatic persons. Samples from symptomatic and AIDS patients showed significantly higher viral excretion rates than samples from asymptomatic patients (12% vs. 0%; $P = 0.0492$).

Cryptosporidium spp. was detected in 49.2% (29 of 59) of the specimens examined, but was not more likely to be present in patients with diarrhea than in patients without diarrhea (48.4% vs. 50%). Except for one adenovirus positive sample, all virus positive samples also contained cryptosporidia.

DISCUSSION

Using several diagnostic assays, we studied the prevalence of acute enteric viruses in fecal samples col-

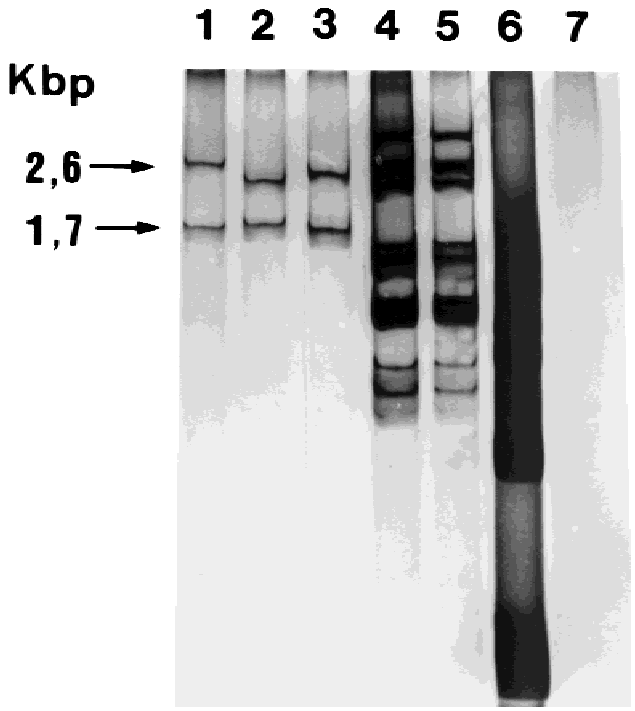

Fig. 1. Electrophoretic pattern of PBV strains identified in fecal specimens from HIV positive patients (lanes 1, 2, and 3). Samples in lanes 1, 2, 3, 5, and 7 were treated with DNase I. Rotavirus double-stranded RNA (lanes 4 and 5) and phage λ DNA digested with *Hind*III (lanes 6 and 7) were used as control substrates for the enzyme and size markers. Gel (7% acrylamide) was stained with silver nitrate.

lected from 125 HIV seropositive patients. We found two different enteric viruses in a total of 6.4% of the patients studied. However, the detection rate in samples from HIV seropositive patients without diarrhea was higher than in patients with diarrhea (8.3% vs. 2.4%). These results are at variance with those reported by several authors [Cunningham et al., 1988; Albrecht et al., 1993; Grohmann et al. 1993; Schmidt et al., 1996] who found that specimens from HIV-infected patients with diarrhea were more likely than those from patients without diarrhea to have enteric viruses. Even though the prevalence of adenovirus found in this study (4%) is comparable with those reported by Grohmann et al. [1993] and Schmidt et al. [1996] (5.8% and 6.6%, respectively), only one of the five adenovirus positive samples detected came from a patient with diarrhea. On the other hand, our results agree with those of earlier studies conducted in the United States [Laughon et al., 1988; Kaljot et al., 1989; Jannof et al., 1991], Africa [Thea et al., 1993], and Europe [Durepaire et al., 1995; Khoo et al., 1995], which fail to show an association between enteric viruses and the diarrhea affecting HIV-infected patients. Table II summarizes published studies of prevalence of enteric viruses in HIV-infected individuals. Despite differences in study design and methodologies, these studies suggest that the relative importance of enteric viruses as etiologic agents of diarrhea in HIV-infected patients may vary according to the epidemiologic settings.

The observation that enteric viruses tend to be more frequently seen in the more advanced stages of HIV infection corroborates results from previous studies comprising different viruses [Cunningham et al., 1988; Kaljot et al., 1989; Jannoff et al., 1991; Thea et al., 1993; Durepaire et al., 1995; Khoo et al., 1995; Schmidt et al., 1996]. This tendency has been observed regardless of an absent or a positive association between virus excretion and diarrhea, and may reflect the superinfections characteristic of late HIV disease stages.

The absence of rotavirus infection in the patients included in this study confirms the marked geographic variation in the prevalence of this agent in HIV seropositive patients. Rotaviruses have been absent or detected at very low rates in studies conducted in America, but have been frequently found in studies conducted elsewhere (Table II). The reason for this geographic variation is unclear.

In the only other study of this nature where astroviruses were considered [Grohmann et al., 1993], they were the most common agent found and were significantly associated with diarrhea. No astroviruses were detected in this study. The possibility of false negatives due to inhibition of the RT-PCR can not be rule out for some samples; however, the nucleic acid extraction protocol used [Boom et al., 1990] is reported in the literature as one of the most efficient in the removal of nonspecific inhibitors for the PCR [Buesa et al., 1996; Hale et al., 1996].

The negative results observed for Norwalk virus in this study cannot be extrapolated to other members of the calicivirus family. For this study, stool samples were tested with a new antigen EIA using hyperimmune antisera from animals immunized with self-assembled Norwalk virus (NV) particles expressed from a recombinant baculovirus [Jiang et al., 1995]. This EIA shows high sensitivity and specificity for Norwalk virus antigen detection, and fails to recognize other small round-structured viruses (SRSV) serologically different from Norwalk virus [Graham et al., 1994; Jiang et al., 1995].

Picobirnaviruses are a group of viruses that have been identified in the feces of several species of vertebrates, including humans [Pereira et al., 1988; Ludert and Liprandi, 1993; Gallimore et al., 1995a]. Grohmann et al. [1993] found an association between PBV and diarrhea in HIV-infected patients. Our results showed a 2.4% prevalence for PBV in the population studied but only samples from patients without diarrhea were found positive. Further studies are required to clarify the role of PBV in the etiology of diarrhea.

Gallimore et al. [1995b] detected PBV in high prevalence in human samples also containing oocysts of *Crytosporidium.* The evidence indicating that PBV are viruses of vertebrates is circumstantial [Pereira, 1991], and PBV may instead be viruses of microorganism. All three samples positive for PBV in this study were also positive for *Crytosporidium.* However, we failed in our attempts to detect PBV genomes in oocysts of *Cryto-*

TABLE I. Prevalence of Enteric Viruses According to Different Centers for Disease Control (CDC) Stages of HIV Infection

CD4+ T-cell categories	Clinical categories A (asymptomatic)	B (symptomatic)	C (AIDS)	TOTAL
	Number of virus/number patients (%)			
1 (≥500 μl)	0/7	0/2	0	0/9[a]
2 (200–449 μl)	0/14	1/15 (6.7)	0/2	1/31 (3.2)[a]
3 (<200 μl)	0/8	3/11 (27.3)	4/37 (10.8)	7/56 (12.5)[a]
Total	0/29[b]	4/28 (14.3)[b]	4/39 (10.3)[b]	8/96 (8.3)

[a]$P = 0.0806$.
[b]$P = 0.0492$.

TABLE II. Prevalence of Enteric Viruses in HIV Positive Patients in Various Studies

Study	Location	Cases/controls	Methods[a]	Agents[b] (%)	Association with diarrhea
Cunningham et al. [1988]	Sydney, Australia	68/55	EIA, IME, EM, culture	RV(19), Ad(15), HCV(1), NV(1)	yes
Laughon et al. [1988]	Washington, United States	49/28	EIA	RV(3), Ad(0)	no
Kaljot et al. [1989]	New York, United States	96/57	EIA, IME, PAGE	Ad(5), SRSV(2), RV(0)	no
Janoff et al. [1991]	Maryland, United States	67/10	EM, culture	Ad(7)	no
Thea et al. [1993]	Kinshasa, Zaire	114/0	EM, EIA	RV(10), SRSV(4), Ad(1), HCV(2)	no
Grohmann et al. [1993]	Atlanta, United States	109/113	EM, PAGE, EIA, PCR	AV(7), PBV(6), Ad(6), CV(3), HCV(2), RV(0)	yes
Albretch et al. [1993]	Hamburg, Germany	66/35	EIA	RV(14)	yes
Khoo et al. [1995]	Manchester, United Kingdom	33/30	EM, culture	Ad(27), RV(3), CV(2)	no
Durepaire et al. [1995]	Limoges, France	35/68	EM, culture	Ad(9)	no
Schmith et al.[1996]	Berlin, Germany	136/120	EM	Ad(7), HCV(11)	yes
Present study	Caracas, Venezuela	41/84	EIA, PAGE, PCR	Ad(4), PBV(2), RV(0), NV(0), AV(0)	no

[a]EIA denotes enzyme immunoassay; EM, electron microscopy; IME, immune electron microscopy; PAGE, polyacrilamide gel electrophoresis; PCR, polymerase chain reaction.
[b]Ad denotes adenovirus; AV, astrovirus; CV, calicivirus; HCV, human coronavirus; NV, Norwalk virus, PBV, picobirnavirus; RV, rotavirus; SRSV, small round-structured viruses.

sporidium concentrated from the PBV positive samples (data not shown).

The results from this and other studies on the whole, suggest that enteric viruses may be second to protozoal and bacterial pathogens as cause of diarrhea in HIV-infected patients [Smith, 1992; Mayer and Wanke, 1994; Shah and Kotler, 1995; Jannof and Smith, 1996; Sharpstone and Gazzard, 1996]. Recent studies have shown that the presence of antibodies seems to be the principal factor that mediates protection from rotavirus reinfection [Franco and Greenberg, 1995; McNeal et al., 1995], and presumably to others acute enteric viruses as well. It has been suggested that patients with AIDS retain the ability to produce antibodies to microorganism encountered before HIV infection [reviewed by Janoff, 1992]. Therefore, while impairment of cellular immunity as HIV disease progresses may account for the increased susceptibility of these patients to infections by protozoal and bacterial pathogens and to reactivation of latent viral infections, the presence of specific antibodies in the mucosal surfaces might explain the relative immunity to enteric viruses observed in HIV-infected patients.

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